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Published in:
The Journal of Physical Chemistry B

DOI:
[10.1021/jp107683d](https://doi.org/10.1021/jp107683d)

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2011

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Citation for published version (APA):

Schrader, T. E., Cordes, T., Schreier, W. J., Koller, F. O., Dong, S-L., Moroder, L., & Zinth, W. (2011). Folding and Unfolding of Light-Triggered beta-Hairpin Model Peptides. *The Journal of Physical Chemistry B*, 115(18), 5219-5226. <https://doi.org/10.1021/jp107683d>

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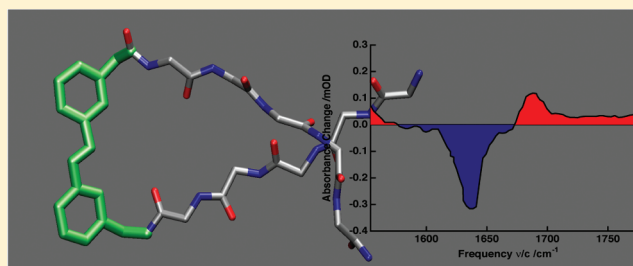
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Folding and Unfolding of Light-Triggered β -Hairpin Model PeptidesTobias E. Schrader,^{†,§} Thorben Cordes,^{†,||} Wolfgang J. Schreier,[†] Florian O. Koller,[†] Shou-Liang Dong,^{‡,⊥} Luis Moroder,[‡] and Wolfgang Zinth^{*,†}[†]Ludwig-Maximilians-Universität München, Lehrstuhl für BioMolekulare Optik and Munich Center for Integrated Protein Science CIPS^M, Oettingenstrasse 67, 80538 München, Germany[‡]Max-Planck-Institut für Biochemie, Am Klopferspitz 18, 82152 Martinsried, Germany

ABSTRACT: Ultrafast spectroscopy in the visible and mid-infrared is used to study the reaction dynamics of two light-triggered model peptides containing an azobenzene derivative as a switching element. One model peptide, the AzoTrpZip2, forms a β -hairpin structure in the *cis* form of the chromophore. This peptide is compared to the core structure consisting of the chromophore and the two flanking amino acid residues, used as a minimal model. This combination of experiments performed in different spectral ranges on peptides of different sizes allows for improved insight into light triggered reaction dynamics. The kinetics observed for the core structure are directly connected to the switching process of the chromophore and are finished on the 10 ps time scale. The *trans*-to-*cis* reaction of AzoTrpZip2, leading to the formation of the β -hairpin structure involves ultrafast processes on the 100 ps time scale, which are directly related to the relaxation of the strain between the isomerized molecular switch and the two peptide strands. IR-signatures characteristic for changes in interstrand interactions are absent on the <1 ns time scale. Thus folding into the β -hairpin structure occurs on a much longer time scale. In the *cis*-to-*trans* unfolding reaction, all IR signatures related to changes in interstrand interactions occur within 1 ns, in a time range where visible spectroscopy reveals the final decay of the intramolecular strain. Apparently unfolding of AzoTrpZip2 is to a large extent a fast, driven process.



1. INTRODUCTION

During folding of proteins, amino acid chains undergo highly complex conformational transitions that cannot adequately be described by present theoretical techniques (e.g., density functional theory or all-atom molecular dynamics simulations). For large proteins, realistic simulations and predictions are prevented by the high dimensionality of the conformational space and by the different time scales of folding, extending from picoseconds up to seconds. A new insight is expected from model systems where experimental and theoretical investigations can be performed with high precision. At present, this is only expected for model peptides of limited size (~ 10 amino acids), where simulations with statistical significance can be combined with high-precision experimental studies. The combination will allow one to address and understand important aspects of protein folding in terms of the formation of secondary structural elements such as turns, helices, and β -sheets. Experiments on small peptides can also provide the experimental foundation for a critical test of the simulation techniques.^{1–3}

In order to obtain information on the rapid initial steps of protein folding, experimental techniques have to be applied which allow one to monitor folding dynamics with ultrahigh temporal resolution. For this purpose, techniques had to be established that combine (i) sufficiently high time resolution in the observation (ultrafast pump probe spectroscopy) with (ii) the ultrafast initiation of the structural changes via photoswitchable peptides, where a photochemically active dye molecule is incorporated as a photoresponsive element.³

A number of photochromic compounds with large-scale structural changes have been proposed in the past as switches for model peptides.^{4–9} Among them azobenzene derivatives have found the most widespread use because of their high switching speed and good photochemical quantum efficiency. In addition, upon photoisomerization the structure of the azobenzene chromophore changes significantly on the time scale of a few hundred femtoseconds.^{10,11} During photoisomerization of the chromophore, the induced changes of its spatial structure exert strong forces, which are exploited for switching conformational preferences of model peptides. For this purpose, azobenzene has been incorporated as a side chain-clamp to photomodulate the folding/unfolding of α -helices^{2,5,12} and collagen-like triple helices^{13,14} in model peptides for studying related kinetics. Similarly, azobenzene derivatized as ω -amino acid has been used as backbone residue within cyclic peptides^{7,15–18} to switch conformational states of turn-like structures. More recently, attention has been focused on the photomodulation of β -hairpins as relatively small supersecondary structures.^{19–21}

Although the β -sheet represents one of the most common structural elements of proteins, it was only with the discovery of

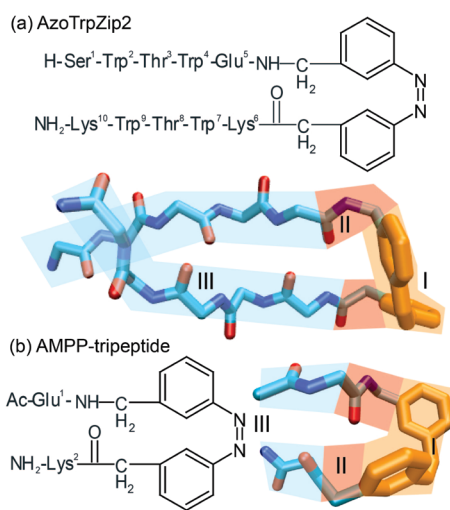
Special Issue: Shaul Mukamel Festschrift

Received: August 13, 2010

Revised: November 10, 2010

Published: December 22, 2010

Scheme 1. Amino Acid Sequence and Typical NMR Structures of (a) AzoTrpZip2 and (b) the AMPP-Tripeptide^a



^a The substitution pattern of the azobenzene in the meta-positions has been found to resemble a β -turn when the azobenzene adopts its *cis* conformation. The AzoTrpZip2 adopts a β -hairpin structure when the AMPP switch is in its *cis* form. For further discussion, we marked the regions of the switching AMPP chromophore (orange, I), the connecting flexible linkers (red, II), and the peptide moieties (blue, III) of both molecules.

folded, monomeric, water-soluble β -hairpins that more detailed knowledge of the particular properties of the turn and strand sequences was obtained, which influence folding and stability of β -sheet structures.^{22–28} The turn sequence important for β -hairpin nucleation often consists of the dipeptide unit Asn-Gly as the statistically most common turn element in crystal structures.²⁹ This peptide sequence was successfully replaced by various non-peptide elements to induce the antiparallel pairing of the two strands and to stabilize the fold in model β -hairpins.^{30–32} These findings encouraged the introduction of the chromophore azobenzene as a turn element to design photoswitchable β -hairpins. Modeling studies have suggested (3-(3-aminomethylphenylazo)-phenylacetic acid (AMPP) as the most suitable derivative to mimic a β -turn when the chromophore is in the *cis* conformation.³³ Moreover, the two methylene spacers between the chromophore and the linking amino and carboxy groups were expected to allow arrangement of the two flanking peptides for interstrand interactions as required for stabilization of the β -hairpin fold. Indeed, by replacing the D-Pro-Gly segment in a 12-residue hairpin derived from the GB1 protein³⁴ with AMPP, photomodulation of the hairpin was successful.¹⁹ Similarly successful was the use of AMPP for substitution of the turn dipeptide Asn-Gly in the well-established tryptophan-zipper (TrpZip) model peptide.^{24,25,35,36} The resulting azobenzene-peptide (derived from TrpZip2) is shown in Scheme 1. This molecule is constructed by three types of building blocks: The molecular switch AMPP (I, orange) is connected via flexible linking groups (II, red, formed by the methylene spacer and the first amide group) with the remaining peptide moiety (III, blue). In this peptide, the azobenzene switch enables the photocontrolled folding and unfolding of the β -hairpin. Indeed, as shown by NMR, the peptide folds into β -hairpin conformations as long as the azobenzene switch is in its *cis* form.²⁰ This conformation is stabilized by hydrogen bonds between the two peptide β -strands and by hydrophobic interactions

between aromatic residues. Upon *cis*-to-*trans* isomerization, the geometry of the switch in the *trans* form prevents a likewise tight coupling between the N- and C-terminal strands of the peptide and the peptide unfolds into an ensemble of structures with little interactions between the two strands. The unfolding dynamics of AzoTrpZip2 triggered by the *cis*-to-*trans* isomerization have been studied by a combination of transient IR-techniques and molecular dynamics simulations. It was found that the unfolding reactions triggered by the *cis*-to-*trans* isomerization of the AMPP switch takes place essentially on the subnanosecond time scale.³⁷ However the folding dynamics initiated by the *trans*-to-*cis* isomerization are much slower and extend out to the ten microsecond time range.

In this paper we present data from transient spectroscopy in the visible and UV of the electronic transitions of the AMPP-switch combined with IR data monitoring the peptide moiety. We compare the results obtained for the β -hairpin model peptide AzoTrpZip2 with those from a minimal AMPP pseudotripeptide consisting of the AMPP chromophore and only one amino acid on either strand (see Scheme 1). We will show that in the visible spectral range the AMPP-tripeptide behaves similarly to a free azobenzene molecule with reaction dynamics finished within a few tens of picoseconds. On the other hand, visible spectroscopy of the AMPP chromophore of AzoTrpZip2 exhibits absorption transients with slower components, which reflect the interactions of the peptide dynamics with the switching chromophore. However, even these absorption changes are completed on the time scale of subnanoseconds, i.e., orders of magnitudes faster than the completion of the folding reaction as deduced from the data in the infrared spectral range. Apparently folding of the β -hairpin model peptide is an allosteric process, where the peptide moiety gradually adjusts in thermal steps to the new structure of the chromophore.

2. MATERIALS AND METHODS

Synthesis and NMR Analysis. The synthesis and NMR conformational analysis of the β -hairpin model peptide AzoTrpZip2 including the molecular switch AMPP has been described previously.²⁰ The AMPP pseudotripeptide Ac-Glu-AMPP-Lys-NH₂ forming the core structure of AzoTrpZip2 will be referred to in the following as the AMPP-tripeptide. It was synthesized by the solid phase procedure applying protocols identical to those reported for the hairpin peptide.²⁰ The homogeneity and structural integrity of the compounds was characterized by analytical HPLC and electrospray ionization mass spectrometry, respectively. The AMPP-tripeptide features an N-terminal acetyl and C-terminal amide group to prevent charge repulsion, whereas the AzoTrpZip2 discussed here bears a free amino terminus and a C-terminal amide (Scheme 1).

The NMR conformational analysis followed the exact same lines as for the hairpin peptide: the same NMR experiments were carried out at similar concentrations and employing the identical NMR spectrometer. However, only very little nuclear Overhauser enhancement (NOE) distance information could be gathered from the spectra. Molecular dynamics calculations were again performed as described for the hairpin peptide.²⁰ Experimental distance constraints expressing conformational turn preferences were only observed for the *cis*-azo isomer of the AMPP-tripeptide.

Stationary Spectroscopy. The peptide samples were dissolved in fully deuterated methanol (Merck, Darmstadt) to yield concentrations of 1.6 mM in the case of the AzoTrpZip2 and 6.4 mM in the case of the AMPP-tripeptide. Stationary spectroscopy was

performed before and after the time-resolved measurements in both the visible and the mid-IR spectral region. In the visible, a Lambda 19 spectrophotometer (Perkin-Elmer) was used, while a Fourier transform infrared (FTIR) spectrometer (IFS66 from Bruker, Ettlingen) recorded the mid-Infrared spectra. In order to switch the azobenzene chromophore of the peptide molecules in solution to its *cis*-isomeric state, a 1000 W HgXe high-pressure lamp (LOT, Darmstadt) was employed, which was equipped with UG1 and WG 320 glass filters (Schott, Mainz). After passing these filters, the light of the HgXe lamp was transferred by an optical fiber to the sample cuvette. On a time scale of minutes, this illumination led to a photostationary state of the *cis*-isomer. The content of *cis*-isomer in that photostationary state was between 80% and 90% (monitored by visible/UV spectroscopy). In order to switch from the photostationary state of the *cis*-isomer to the *trans*-isomer, a cold-light source KL2500 LCD (Schott, Mainz) was used in combination with the filters KG2 and GG400 (Schott, Mainz). Again the light was guided to the sample cell with an optical fiber.

Transient Spectroscopy. Information on reaction dynamics were obtained by pump–probe experiments using ultrashort excitation light pulses (~ 100 fs). The time-dependent absorbance changes were deduced from probing with properly delayed light pulses of different wavelengths.

A home-built Ti-sapphire laser/amplifier system generated ultrashort light pulses (~ 100 fs) centered at 804 nm with 0.66 mJ pulse energy at a repetition rate of 1 kHz.³ The laser fundamental of this chirped pulse amplifier (CPA) was frequency doubled to obtain pump-pulses at 402 nm for the transient absorption experiment. The excitation energy varied from 150 to 800 nJ (visible) and 4 to 12 μ J (IR). For probing in the visible/UV, a small fraction of the laser fundamental at 804 nm was focused into a calcium fluoride crystal, generating a broadband white-light continuum.³⁸ The white light continuum was used to record the pump-induced absorbance changes within a multichannel detection system.³⁹ The probing process covered a spectral range from ~ 350 to 650 nm. The experiments performed with probing in the visible used linearly polarized pump and probe beams with polarizations set at the magic angle of 54.7° . Probing in the IR was performed with parallel and perpendicular polarizations, and the absorption changes expected for magic angle (displayed in the figures) was calculated. Beam diameters in the sample were set to $d_{\text{pump}} = 150 \mu\text{m}$, $d_{\text{probe}} = 50 \mu\text{m}$, full width at half-maximum (fwhm). In order to improve referencing, every second pump pulse was blocked by a mechanical chopper. During the pump–probe measurement, the sample solution was pumped through a flow-cell based on calcium fluoride windows and an optical path length of $220 \mu\text{m}$. The peptide samples were circulated through the cell with a Teflon tubing pump (ISMATEC, Glattbrugg-Zurich, Switzerland) at a speed sufficient to ensure complete sample exchange between two excitation pulses in the time-resolved measurements. In addition, the concentration of *cis*-isomer was kept constant by illuminating the sample with spectrally filtered light from the HgXe lamp described above. The experimental setup used for the measurement of the transient absorption changes in the mid-IR has been presented recently in ref 40.

The data points taken at certain delay times between pump and probe were measured with repetitive scans (ca. 5–15). Each data point consists of ~ 5000 – 15000 averaged single laser shots. Transient background signals from the pure solvent were weighted and subtracted from the sample signal. The temporal

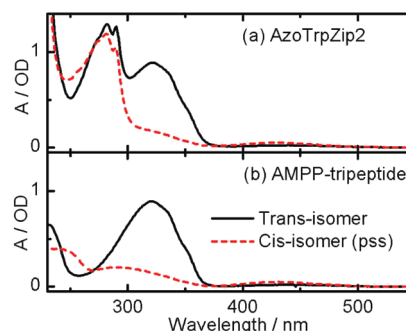


Figure 1. Absorption spectra in the visible spectral region of (a) the AzoTrpZip2 and (b) the AMPP-tripeptide. The spectra with the AMPP switch in the *trans* conformation are drawn with a solid line. To represent the properties of the *cis*-isomers, the spectra of the photostationary state (pss) after illumination (see text) are shown with broken curves. These photostationary states (pss) contain about 85% of *cis*-isomer of the corresponding model peptide.

behavior of all wavelength-dependent transients was modeled by a multiexponential function fitted by a global least-squares algorithm (Levenberg–Marquart). Further details concerning the transient absorption setup are reported in refs 8 and 41.

3. RESULTS

In Scheme 1a, the primary structure of the β -hairpin model peptide AzoTrpZip2 is shown together with its solution structure as derived from NMR conformational analysis of the peptide with the azobenzene chromophore in the *cis* form.²⁰ In this structure, most of the backbone is arranged in a nearly antiparallel fashion in accordance with an ordered β -hairpin. It strongly resembles the β -hairpin structure of the parent TrpZip2 peptide.^{24,25} The turn region of the hairpin is formed by the AMPP chromophore and the two directly attached amino acid residues. As a mimic of this portion I of the β -hairpin peptide, the AMPP-tripeptide was synthesized where the amino and carboxyl groups were acetylated and amidated, respectively, to prevent intramolecular electrostatic interaction between the charged side chains and the terminal groups. The AMPP-tripeptide shown in Figure 1b contains four C=O groups in the backbone and one in the glutamate side chain. Conversely, 12 C=O groups are present in the AzoTrpZip2 peptide where only the C-terminus is protected by amidation.

The NMR experiments on the *cis*-AMPP-tripeptide yielded NOE values which were used to determine model structures. The turn-like structure shown in Scheme 1b is illustrating the typical low energy conformations found for this reference tripeptide in the *cis*-azo-form. For the *trans*-isomer of the AMPP-tripeptide no relevant NOEs were found, allowing no defined structural models to be obtained. However the simple covalent geometry of this isomer does not permit regular turn conformations without additional strain or geometric restrictions.

Stationary Spectroscopy of the Model Peptides. The UV/visible absorption spectra of the two azobenzene peptides AzoTrpZip2 and AMPP-tripeptide are reported in Figure 1. For both peptide samples, prepared by stationary illumination at appropriate wavelengths (see Materials and Methods), the absorption spectra (at $\lambda > 300$ nm) are very similar. This spectral range is related to the azobenzene moiety of the two chromopeptides. The 321 nm absorption band results from the $\pi\pi^*$ -transition of the molecules as *trans*-isomers (solid lines). Around 430 nm, there is

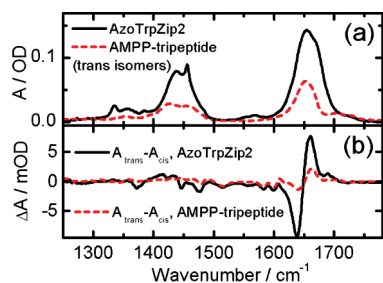


Figure 2. Absorption (a) and difference (b) spectra in the infrared spectral region of the AzoTrpZip2 (solid line) and the AMPP-tripeptide (broken line). The spectra of the two molecules were scaled for the same chromophore concentration and are corrected for the absorption of trifluoroacetic acid.

a weak absorption band caused by the $n\pi^*$ -transition. Here the absorption of the *cis*-isomers (dashed lines) is stronger. Compared to neat azobenzene (data not shown), the *trans* $\pi\pi^*$ absorption band of both azobenzene-peptides is shifted by 6 nm to higher wavelengths and is slightly broadened. Pronounced differences between the two azobenzene-peptides are found at shorter wavelengths, especially between 260 and 300 nm, and these are due to the absorption bands in this spectral region of the Trp residues⁴² present only in the hairpin peptide.

Clear differences between the two peptide samples (normalized to the same azobenzene concentration) show up in the stationary IR-spectra (see Figure 2). Strong IR-absorption bands related to the backbone of the peptide are seen in Figure 2a around 1650 cm^{-1} (amide I') and 1450 cm^{-1} (amide II of the deuterated amide groups). Please note that the absorption peak around 1670 cm^{-1} seen in ref 37 is partially due to traces of trifluoroacetic acid in the sample. In Figure 2 we subtracted this contribution. Weak absorption bands from the azobenzene chromophore in its *cis* form appear between 1450 cm^{-1} and 1600 cm^{-1} . The chromophore bands at 1585 cm^{-1} and 1600 cm^{-1} are clearly visible in the difference spectra presented in Figure 2b. Since the number of amide C=O groups is considerably smaller in the AMPP-tripeptide (broken curves), the corresponding bands (amide I' and II) show significantly lower amplitudes. A rich spectral signature is found when the changes in IR-absorption, induced by photoisomerization of the azobenzene, are recorded (Figure 2b). The AzoTrpZip2 peptide shows a pronounced dispersive line shape in the range of the amide I' band. These isomerization-induced absorption changes have been assigned to the structural differences and related changes in the H-bond pattern. Further bands in the absorption difference spectra are found in the range of 1350 to 1500 cm^{-1} (amide II and chromophore bands) and at 1585 and 1600 cm^{-1} assigned to the azobenzene chromophore. The bands assigned to the chromophore show the same bleach for AzoTrpZip2 (solid line) and the AMPP-tripeptide (dashed line). A dispersive feature in the range of the amide I' band indicates that structural changes also occur in the AMPP-tripeptide. However, the related amplitude is strongly reduced as compared to AzoTrpZip2. The reduction in amplitude is stronger than expected from the smaller number of carbonyl groups in the AMPP-tripeptide.

Ultrafast Spectroscopy in the Visible. An overview of the reaction dynamics of the two model compounds is given in Figure 3, where the transient absorption changes recorded by time-resolved techniques in the visible (380 nm–650 nm) are presented. The first column shows the data for AzoTrpZip2 upon the *trans*-to-*cis* (Figure 3a) and *cis*-to-*trans* isomerization of the azobenzene

(Figure 3b) associated with the hairpin folding and unfolding, respectively. Here data for probing wavelengths $\lambda_{\text{pr}} = 410$ –650 nm are shown. It should be noted that scattering of the sample prevented recording of reliable data at shorter probing wavelengths $\lambda_{\text{pr}} < 410$ nm. Immediately after excitation, there is a strong increase in absorption throughout the whole investigated spectral range for both isomerization directions. This induced absorption decays on the time scale of picoseconds toward a weak offset, which is due to the formation of the photoproducts and which agrees with the respective stationary absorption difference spectra. A modeling of the data with exponential functions shows that the initial absorption changes related to the *trans*-to-*cis* isomerization (Figure 3a) may be fitted by two kinetics of similar amplitudes with time constants $\tau_1 = 3.2$ ps and $\tau_2 = 54$ ps. For the unfolding (*cis*-to-*trans*) reaction, somewhat longer time constants ($\tau_1 = 6.7$ ps and $\tau_2 = 200$ ps) are found.

The absorption dynamics of the AMPP-tripeptide have a similar spectral shape, but accelerated reaction dynamics: For the *trans*-to-*cis* reaction (Figure 3c), we observe a broad initial absorption increase throughout the whole visible and near UV with two bands at 530 nm and <350 nm. The absorption increase decays rapidly on the time scale of 1 ps. The modeling procedure again yields two time constants. Most of the absorption change occurs with $\tau_1 = 1.8$ ps, while a second dynamic process with $\tau_1 = 13$ ps has only small amplitudes in the visible spectral range. The *cis*-to-*trans* reaction (Figure 3d) shows even faster reaction dynamics. Here the initial absorption increase is modulated by ground-state bleach of the $n\pi^*$ band of the original *cis* form of the chromophore. The dominant part of the absorption increase found at early times decays on the subpicosecond time scale ($\tau_1 = 0.2$ ps). An additional component ($\tau_2 = 2.7$ ps) shows a weaker amplitude. At late delay times, one observes the absorption changes known from the stationary illumination experiments. The results demonstrate that similar processes occur as found previously for other azobenzene compounds. For both directions of the photoreaction, the AMPP-tripeptide shows reaction times and absorption difference spectra that are similar to those observed for neat azobenzene chromophores.^{3,11,43} The reaction dynamics of AzoTrpZip2 are characterized by fast picosecond components combined with weaker transients in the 50 to 200 ps time scale. These slower features are known from cyclic and bicyclic (4-aminomethyl)-phenylazobenzoic acid (AMPB) and (4-amino)-phenylazobenzoic acid (APB) peptides, where they are attributed to the long-lasting relaxation of the interaction of the peptide with the azobenzene chromophore.^{3,44}

The difference between the dynamics of the AMPP-tripeptide (filled squares) and AzoTrpZip2 (open circles) are clearly visible in Figure 4, where we plotted the transient absorption changes (normalized) for a probing wavelength of 542 nm. In both samples, we find an excitation-induced absorption increase, which decays subsequently. In the AMPP-tripeptide, a strong and fast decay occurs at early delay times, and the dominant part of the absorption changes disappears within 10 ps. For AzoTrpZip2 the kinetics are much slower. The behavior is very similar to the one observed for other azobenzene peptides, where the initial motion of the azobenzene during the isomerization process is constrained by the peptide moiety.³ The interaction slows down the relaxation to the ground-state of the azobenzene. Subsequently the interaction keeps the molecule in a distorted (ground-state) geometry, which is relaxed only on the 100 ps time scale and which is visible by the long-lasting absorption increase.

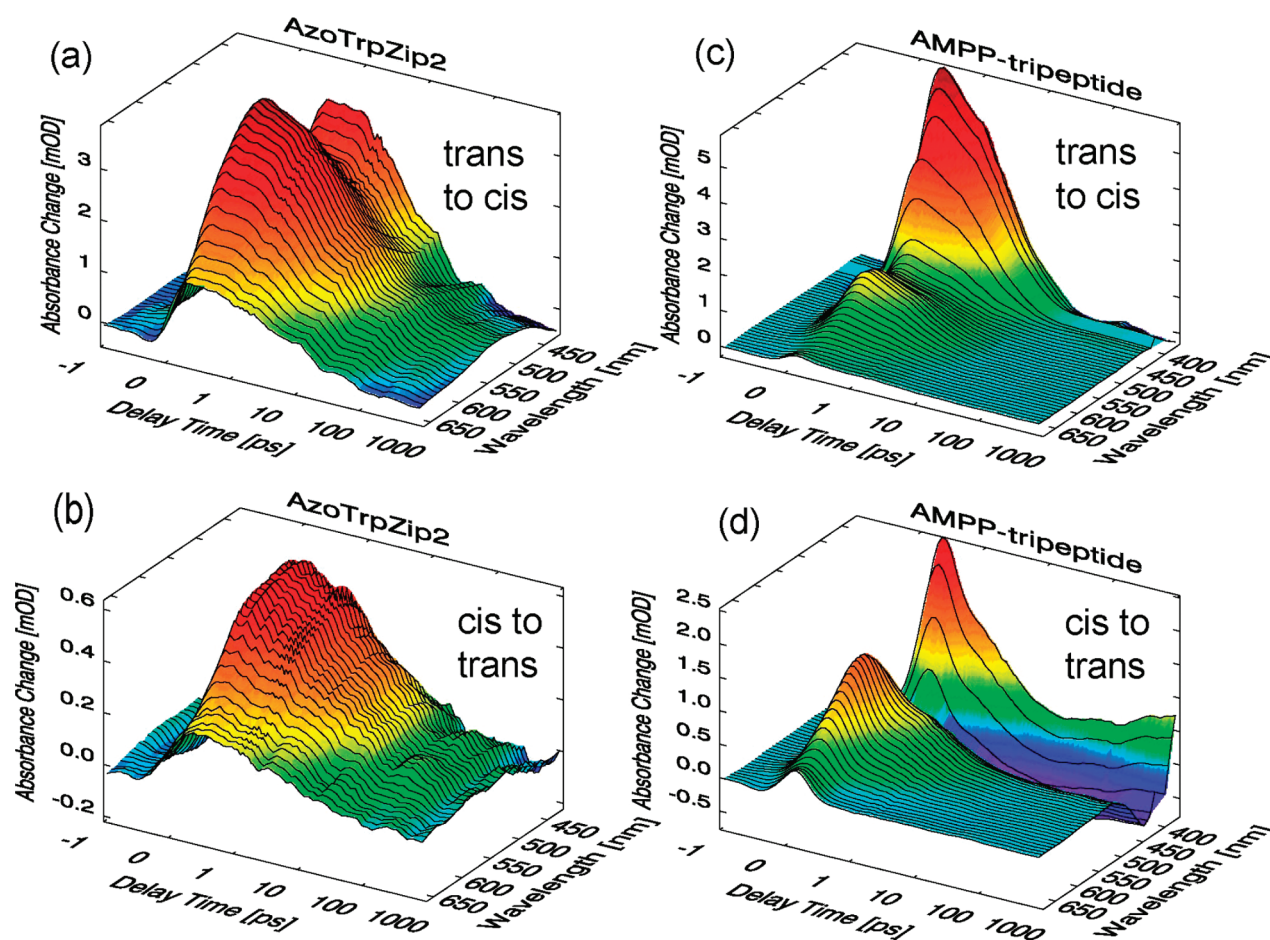


Figure 3. Overview over the time-resolved absorption spectra measured for the AzoTrpZip2 and the AMPP-tripeptide. The color codes the absorbance change. The time axes are linear between -1 and 1 ps and logarithmic thereafter. Left: the data recorded on AzoTrpZip2 with excitation of the *trans*-isomer (a) and *cis*-isomer (b). Right: data sets on the AMPP-tripeptide with excitation of the *trans*-isomer (c) and the *cis*-isomer (d).

The Transient Absorption Changes in the IR . *Cis-to-Trans* Reaction. Figure 5 shows time-resolved IR difference spectra recorded at various delay times for the *cis-to-trans* isomerization of both peptide systems. The left part presents AzoTrpZip2, where difference spectra have been published previously.³⁷ At early delay times (~ 1 ps) just after the population of the excited electronic state of AMPP, there is a strong absorption bleach around 1650 cm^{-1} with a shoulder at 1670 cm^{-1} . A broad increase in absorption at other frequencies may be seen as a signature of an excited electronic state or of a molecular system with considerable vibrational excess energy.⁴⁴ At 1585 and 1600 cm^{-1} a dip in the absorption increase is found, which can be assigned to AMPP *cis* bands that are bleached due to the remaining excitation. During the next picoseconds, the absorption decrease is reduced and red-shifted. There is a pronounced absorption decrease in the spectral range (1640 cm^{-1}) known for strongly hydrogen bonded amide groups.^{45,46} Due to the isomerization of the AMPP chromophore, hydrogen bonds between the two strands are broken, and the rapture continues to evolve on the few picosecond time scale. An absorption increase is found around 1680 cm^{-1} , pointing to the appearance of amide groups in an environment of low dielectric constant. On the time scale of 10 ps, all signatures of vibrational excess energy disappears. Subsequently, in the 20 – 150 ps range, the absorption bleach recovers partially, and the induced absorption around 1680 cm^{-1}

vanishes. Apparently, amide groups and their surroundings re-adjust to a new arrangement, which leads to resolution in the solvent methanol. With a time constant of 630 ps, an absorption increase grows at $\sim 1655\text{ cm}^{-1}$. The absorption difference spectrum now becomes very similar to the stationary absorption difference spectrum (broken curve). Within a few nanoseconds, the AzoTrpZip2 peptide has essentially completed the structural changes related with hairpin unfolding.

At early delay times, the AMPP-tripeptide shows features similar to those of AzoTrpZip2. The main absorption decrease is located at somewhat higher frequencies, and the relative amplitudes in the azobenzene bands are larger. On the time scale of a few picoseconds, a weak red shift of the amide I' bleach occurs. Its peak is now located at 1645 cm^{-1} . This band decays subsequently on the 15 ps time scale, and a weak sigmoidal feature is left after 150 ps. From this time on, the transient absorption change is identical to the stationary difference spectrum (solid curve).

At first glance, the transient IR data for the *cis-to-trans* reaction of AzoTrpZip2 and the AMPP-tripeptide look similar. However, pronounced differences are evident at closer inspection (see Figure 5): (i) The early bleaching of the amide band occurs at higher frequencies in the AMPP-tripeptide sample (1647 versus 1640 cm^{-1}). This indicates that amide groups with stronger hydrogen bonding are involved in AzoTrpZip2. (ii) The relative

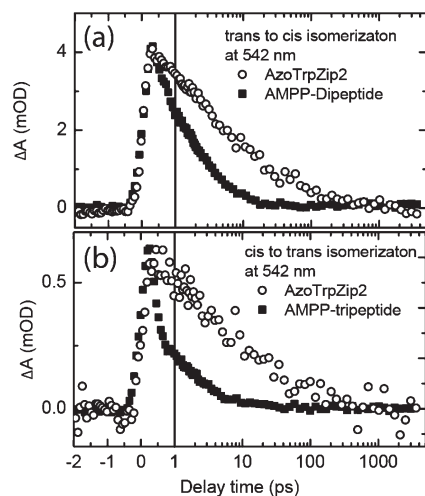


Figure 4. (a) Transient absorption data at a probe wavelength of 542 nm for exciting the *trans*-isomer of the AzoTrpZip2 (open circles) and the AMPP-tripeptide (squares). (b) Transient data at a probe wavelength of 542 nm for exciting the *cis*-isomer of the AzoTrpZip2 (open circles) and the AMPP-tripeptide (filled squares). The time delay axes are linear between -1 and 1 ps and logarithmic thereafter.

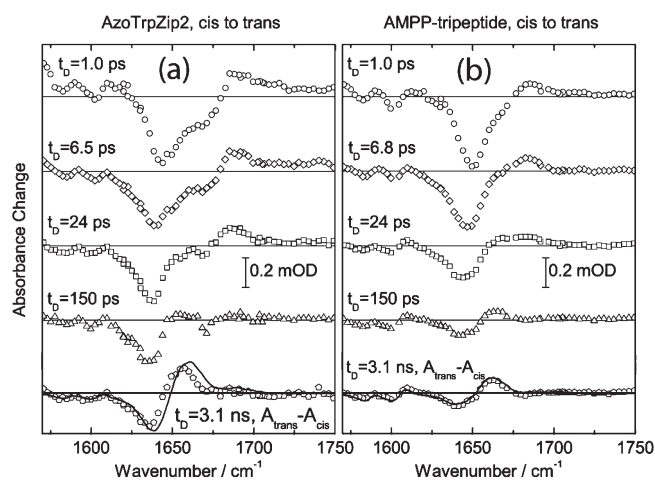


Figure 5. Transient IR spectra (symbols) of the data of the AzoTrpZip2 (a) and the AMPP-tripeptide (b) for the isomerization from *cis* to *trans* and stationary difference spectra (solid lines). The data in panel b is scaled for the same number of excited molecules as in panel a.

changes of the azobenzene versus peptide bands are smaller in AzoTrpZip2, which points to a larger number of amino acids influenced by the isomerization of AMPP. (iii) The disappearance of the absorption band of the desolvated amide groups (1680 cm^{-1}) occurs faster in the AMPP-tripeptide. Here vibrational cooling and resolution are finished within $20\text{--}30\text{ ps}$. (iv) The final absorption transients leading to the stationary absorption difference spectrum are much faster for the AMPP-tripeptide. The experimental data demonstrate that the tripeptide construct behaves, as expected from its small size, similarly to the pure azobenzene chromophore.³ On the other hand, AzoTrpZip2 shows pronounced contributions at longer times, representing peptide chromophore interactions as well as pure peptide dynamics.

Trans-to-Cis Reaction. Experimental data for the *trans*-to-*cis* reaction are shown in Figure 6. Both samples show similar

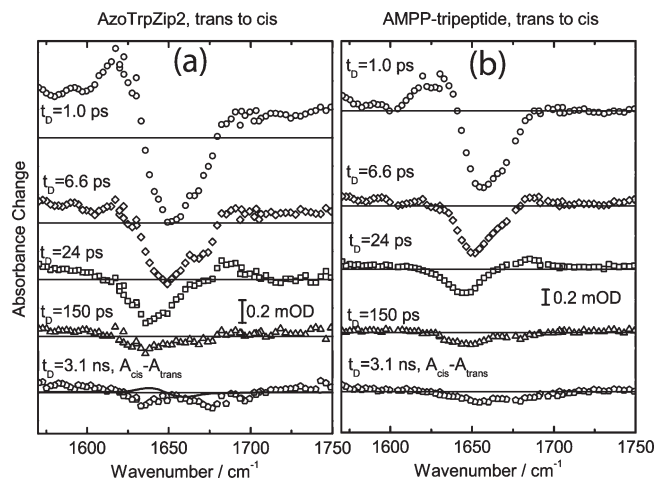


Figure 6. Transient IR spectra (symbols) of the AzoTrpZip2 (a) and the AMPP-tripeptide (b) for the isomerization from *trans* to *cis*. The data in panel b is scaled for the same number of excited molecules as in panel a.

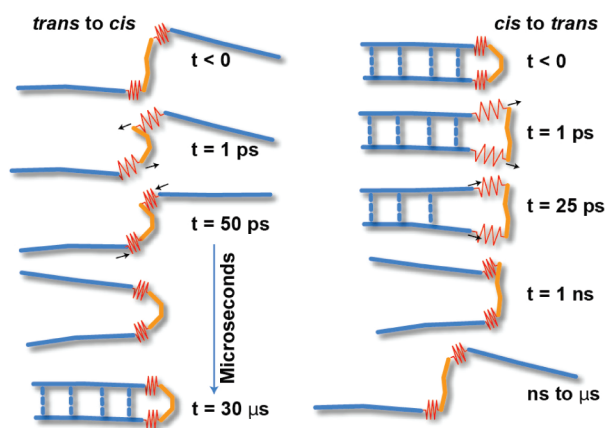
absorption features. Immediately after excitation, there is a strong bleach of the amide I' band around 1655 cm^{-1} together with some absorption increase in its red wing. Subsequently, all absorption changes decrease. After 24 ps , the absorption bleach is shifted to 1645 cm^{-1} , and the amplitudes continue to decay. For AzoTrpZip2, absorption changes remaining after 3 ns do not resemble the stationary absorption difference spectrum. These differences point to subsequent rearrangements of the peptide moiety on a much longer time scale.³⁷ The experiments convincingly show that the dominant part of the absorption changes for both samples upon the *trans*-to-*cis* reaction can be assigned to photoinduced processes in the AMPP chromophore, the directly attached amino acids, and the solvent surroundings. This finding is not unexpected considering the low isomerization yield of the *trans*-to-*cis* photoreaction of azobenzene. The distinct features assigned in the case of the *cis*-to-*trans* reaction to rearrangements of the peptide moiety are missing. They occur on a much longer time scale.

4. DISCUSSION

The time-resolved experiments on the AzoTrpZip2 hairpin and its core structure, the AMPP-tripeptide, reveal similarities as well as pronounced differences, which can be used to gain new insight into the reaction dynamics. In order to visualize the structural changes involved, we use the simplified picture given in Scheme 2, where the transient structures at a number of delay times are represented schematically for the *trans*-to-*cis* (left) and the *cis*-to-*trans* reaction (right). In both situations, the rapid isomerization of the AMPP switch with its large geometric changes exerts forces on the other parts of the molecule. They are transferred via the linkers (part II in Scheme 1) to the peptide moiety. The flexibility of the linkers (represented in Scheme 2 by the strings) may enable the isomerization of the switch even without movements in the peptide part. Subsequently, the (strong) strain may initiate structural changes of the peptide in close vicinity of the linkers followed by large scale motions of the peptide. On the subnanosecond time scale, the energy stored in the strain approaches thermal energy, and allosteric rearrangements should guide the stationary ensemble of peptide structures.

AMPP-Tripeptide. The reaction dynamics mainly reflect the switching of the AMPP chromophore. The time constants observed

Scheme 2. Schematic Representation of the Structural Intermediates in the *Trans* to *Cis* (Left) and *Cis* to *Trans* (Right) Reaction of AzoTrpZip2^a



^a The structural changes related to the isomerization of the AMPP switch (orange) induced strain on the peptide part (blue), which were transferred by the flexible linker (red spring). Only in the case of the *cis* to *trans* reaction so ultrafast changes in the inter-strand interactions occur, which are observed in the transient IR spectra. For the *trans* to *cis* reaction, the ultrafast processes involve relaxations within AMPP, linkers, and peptide moiety, without the change of interstrand interactions.

in the visible and UV are similar to the ones observed previously for neat azobenzene. The transient IR experiments show that rearrangements of the two amino acids occur on the 10 ps time scale directly following the isomerization of the AMPP switch. In addition there are diffusive rearrangements of the amino acids and the transfer of vibrational excess energy from the AMPP-tripeptide to the surrounding solvent. In the AMPP-tripeptide, the small size of the peptide groups attached to the linkers leads to a release of the strain on the time scale of isomerizational motion and vibrational energy dissipation.

AzoTrpZip2 *Trans*-to-*Cis*. The *trans*-to-*cis* reactions of the AzoTrpZip2 and the AMPP-tripeptide show the same type of absorption transients. The qualitative similarity of the IR data of the two compounds allows one to interpret the ultrafast absorption changes. It suggests that the isomerization of the AMPP switch influences predominantly the structural arrangement of adjacent amino acids (the linkers, represented in Scheme 2 by the springs). For the *trans*-to-*cis* reaction of AzoTrpZip2, small-scale adjustments of the dihedral angles of the backbone in close vicinity to the AMPP allow a rapid relaxation (time constant of 50 ps, seen in the visible absorption experiments) of the strain between peptide moiety and AMPP chromophore. No indications are found in the IR data for changes in the interstrand interactions. This is not unexpected since large-scale ultrafast structural changes are not expected when starting with AzoTrpZip2 in the *trans* form, where (according to NMR structure analysis²⁰) no interstrand hydrogen bonds exist close to the AMPP chromophore. Only on a much longer time scale do diffusive processes lead to the adjustment of the whole peptide to the new AMPP configuration. The time scale of these allosteric processes extends into the microsecond range.³⁷ Whether an energy barrier is encountered in these processes can only be investigated by measuring the temperature dependence of these slower folding processes.

AzoTrpZip2 *Cis*-to-*Trans*. The dynamics of the AzoTrpZip2 for the *cis*-to-*trans* unfolding reactions investigated by IR spectroscopy

have been treated in detail in the literature.³⁷ The additional information from the time-resolved visible spectroscopy allows us to add new details for the understanding of the unfolding reaction. In the case of the compact arrangement of the amino acids in the β -hairpin structure with several interstrand hydrogen bonds, AMPP isomerization causes not only the stretching of the linkers but also a rapid breaking of hydrogen bonds on the time scale of a few picoseconds. Even after the relaxation of excess vibrational excitation on the 20 ps time scale, the dense packing of the β -hairpin structure still remains, which continues to impose constraints on the isomerized AMPP chromophore. The relaxation of these constraints (observed via the 200 ps decay process in visible spectroscopy; see Figure 3) is directly related to the final (630 ps) transient observed in the IR. The visible absorption changes suggest that these final unfolding processes may be driven by forces between AMPP and the peptide moiety. Apparently, the closely packed and hydrogen bonded β -hairpin structure of *cis*-AzoTrpZip2 restricts relaxation motions of the dihedral angles and bond rotation in the backbone and does not allow the complete relaxation of the strain between the peptide moiety and the AMPP chromophore on the sub-100 ps time scale. Since the visible and IR absorption changes reached after 1 ns are very similar to the stationary difference spectra, the remaining structural rearrangements are not driven by the intramolecular strain. These motions should leave the structure of the strands and the interactions between them essentially unaffected.

In conclusion, we have presented an experimental study of the initial reaction dynamics of two azobenzene model peptides combining visible and IR spectroscopy. Using the AMPP-tripeptide as a minimal model, we could determine the absorption changes directly related to the AMPP chromophore and its direct surrounding for both isomerization directions. Within less than 10 ps we find fast structural changes of the AMPP chromophore. The rearrangement of the two amino acids occurs on the same time scale. For the initial steps of the folding reaction of AzoTrpZip2 triggered by the *trans*-to-*cis* photoisomerization of the AMPP chromophore, we also observe a fast rearrangement related with a rapid relaxation of the strain between peptide and AMPP chromophore within 50 ps. The IR spectra do not show any indication of the completion of the folding process on the time scale of 1 ns. Since the intramolecular strain and vibrational excess excitation is released on the subnanosecond time scale, folding must occur in much slower, thermally driven processes, where the peptide structure adjusts to the *cis* AMPP loop region in allosteric reaction steps. On the other hand, the tightly packed AzoTrpZip2 β -hairpin molecule is rapidly unfolded by the *cis*-to-*trans* photoisomerization of the AMPP within about 1 ns.

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■ ACKNOWLEDGMENT

The authors thank Alexander Milbradt, Christian Renner, and Markus Löweneck for synthesis of the molecules and NMR analysis, and Ulrike Kusebauch for helpful discussions. Financial support from the Deutsche Forschungsgemeinschaft (SFB 533 Projects A8, B9, and SFB 749, project A5) is highly acknowledged. This work was also supported through the DFG-Cluster of Excellence Munich—Centre for Advanced Photonics. The authors thank Shaul Mukamel for many helpful discussions on light triggered peptides.

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